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		DB=USPT,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L10	L7 and liposome$	143
☐	L9	L7 and 424/450.ccls.	4
☐	L8	L7 and 424/450/ccls.	0
☐	L7	(enzyme$ or protein$) adj5 (glycerol)	1066
☐	L6	L5 and liposome$	25
☐	L5	(enzyme$ or protein$) same (glycerol adj5 stabili$)	196
☐	L4	(enzyme$ or protein$) same (glycerol adj5 stabili$)	196
☐	L3	L2 and (glycerol adj5 stabili$)	20
☐	L2	liposomes same (enzyme$ or protein$)	9159
☐	L1	liposomes same (enzyme$ or protein$)	9159

END OF SEARCH HISTORY

L10: Entry 34 of 143 File: USPT Oct 1, 2002

DOCUMENT-IDENTIFIER: US 6458387 B1
TITLE: Sustained release microspheres

Brief Summary Text (9):
The most common method of covalently binding an antibody to a solid phase matrix is to derivative a bead with a chemical conjugation agent and then bind the antibody to the activated bead. The use of a synthetic polymeric bead rather than a protein molecule allows the use of much harsher derivatization conditions than many proteins can sustain, is relatively inexpensive, and often yields a linkage that is stable to a wide range of denaturing conditions. A number of derivatized beads are commercially available, all with various constituents and sizes. Beads formed from synthetic polymers such as polyacrylamide, polyacrylate, polystyrene, or latex are commercially available from numerous sources such as Bio-Rad Laboratories (Richmond, Calif.) and LKB Produkter (Stockholm, Sweden). Beads formed from natural macromolecules and particles such as agarose, crosslinked agarose, globulin, deoxyribose nucleic acid, and liposomes are commercially available from sources such as Bio-Rad Laboratories, Pharmacia (Piscataway, N.J.), and IBF (France). Beads formed from copolymers of polyacrylamide and agarose are commercially available from sources such as IBF and Pharmacia. Magnetic beads are commercially available from sources such as Dynal Inc. (Great Neck, N.Y.).

Brief Summary Text (13):
Microparticles prepared using lipids to encapsulate target drugs are currently available. For example, lipids arranged in bilayer membranes surrounding multiple aqueous compartments to form particles may be used to encapsulate water soluble drugs for subsequent delivery as described in U.S. Pat. No. 5,422,120 to Sinil Kim. These particles are generally greater than 10 .mu.m in size and are designed for intra articular, intrathecal, subcutaneous and epidural administration. Alternatively, liposomes have been used for intravenous delivery of small molecules. Liposomes are spherical particles composed of a single or multiple phospholipid and cholesterol bilayers. Liposomes are 30 .mu.m or greater in size and may carry a variety of water-soluble or lipid-soluble drugs. Liposome technology has been hindered by problems including purity of lipid components, possible toxicity, vesicle heterogeneity and stability, excessive uptake and manufacturing or shelf-life difficulties.

Drawing Description Text (8):
FIG. 7 is a bar graph showing the amount of expressed gene product by beta - galactosidase activity in milliunits versus microsphere formation for naked DNA, cationic liposomes containing DNA, and DNA microspheres.

Detailed Description Text (23):
When preparing microspheres containing protein, a protein stabilizer such as glycerol, fatty acids, sugars such as sucrose, ions such as zinc, sodium chloride, or any other protein stabilizers known to those skilled in the art may be added prior to the addition of the polymers during microsphere formation to minimize protein denaturation.

Detailed Description Text (174):
The uptake and expression of the pCMV beta Gal DNA was assayed for efficiency of

transfection and amount of expressed beta -galactosidase enzyme. The efficiency of transfection was determined by fixation of the cells and color development with the beta-galactosidase enzyme substrate X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside, GIBCO-BRL, Gaithersburg, Md.). The amount of expressed beta-galactosidase enzyme was determined by lysing the transfected cells and measuring total enzyme activity with the beta -galactosidase enzyme substrate CPRG (chlorphenolred-beta-D-galactopyranoside, Boehringer Mannheim, Indianapolis, Ind.) Results: The amount of expressed beta -galactosidase enzyme from lysed cells that were transfected using either: 1) naked DNA (no addition); 2) cationic liposomes plus DNA; or 3) DNA-containing microsphere, prepared as described above, is shown in FIG. 7.

L10: Entry 47 of 143 File: USPT Sep 25, 2001

DOCUMENT-IDENTIFIER: US 6294365 B1
TITLE: Method and formulation for stabilization of enzymes

Brief Summary Text (8):
Excipients are inert substances that give a desired characteristic to solvents. Examples of such excipients include sugars, glycerol, polyethylene glycols, amino acids, and other osmolytes. Certain hydrophilic excipients confer stability by making the solvent more polar. An increase in solvent polarity results in an increase in the free energy of transfer for the hydrophobic amino acid moieties from within the protein to the solvent thereby making it more difficult for the protein to unfold (Alonso and Dill, 1991). Glycerol is a common excipient used by enzymologists for storage of enzymes at low temperatures (-20.degree. C.), since some enzymes stored frozen in 50% glycerol buffers may retain much of their initial activity for years.

Brief Summary Text (12):
The use of sugars such as the disaccharide trehalose as stabilizing agents has been used in conjunction with lyophilization to stabilize polysaccharides (Guthohrlein and Helting, European Patent No. GB 2 009 198 A) and liposomes (Crowe et al., 1987). Lyophilization has also been used to stabilize tumor necrosis factor in the presence of a nonionic surfactant and sugars such as trehalose (Hayashi and Komiya, European Patent No. GB 2 126 588 A). Enzymes that have been stabilized with sugars during lyophilization include phosphofructokinase (Carpenter et al., 1987), which was stabilized with glucose, galactose, maltose, sucrose, and trehalose, and alkaline phosphatase (Ford and Dawson, 1992) which was stabilized with mannitol, lactose, and trehalose.

Detailed Description Text (5):
The purified enzymes that were used to study potential stabilizing excipients were T7 RNA Polymerase, Avian Myeloblastosis Virus-Reverse Transcriptase (AMV-RT) and RNase H (Molecular Biology Resources, Milwaukee, Wis.). These enzymes were stored in 50% glycerol storage buffers at -20.degree. C. or -70.degree. C., since glycerol lowers the freezing point of proteins closer to the storage temperature. Before lyophilization, it was necessary to remove glycerol because it acts as a hygroscopic agent and the enzyme preparations would not lyophilize. Each enzyme was individually deglycerolized by ultrafiltration into its appropriate storage buffer containing no glycerol as described below.

Detailed Description Text (9):
To deglycerolize the enzymes, an appropriate volume of enzyme (in glycerol) was added to each concentrator. The amount that was deglycerolized was determined by the number of units required in the final lyophilization. Generally, an amount from 50-150 .mu.L enzyme in glycerol buffer was added to the prepared concentrators. The appropriate buffer without glycerol was added to obtain a volume of about 10 times the initial enzyme volume (up to 1.5 ml total) and the two phases were thoroughly mixed by pipetting, being careful not to disrupt the filter. It is important to avoid overdilution of the enzymes with the non-glycerol buffer, since this may contribute to activity loss. Each concentrator was centrifuged in an SA-600 rotor at 5000.times.g for approximately 45 minutes. The process was repeated twice.

Detailed Description Text (63):
Crowe, J. H., B. J. Soargo, and L. M. Crowe. Preservation of dry liposomes does not require retention of residual water. Proc. Natl. Acad. Sci., 84: 1537-1540 (1987).

Other Reference Publication (5):
Crowe et al., "Preservation of Dry Liposomes Does Not Require Retention of Residual Water," Proc. Natl. Acad. Sci., 84:1537-1540 (Mar., 1987).

L10: Entry 53 of 143 File: USPT Jul 31, 2001

DOCUMENT-IDENTIFIER: US 6268053 B1
TITLE: Macromolecular microparticles and methods of production and use

Brief Summary Text (7):
The most common method of covalently binding an antibody to a solid phase matrix is to derivatize a bead with a chemical conjugation agent and then bind the antibody to the activated bead. The use of a synthetic polymeric bead rather than a protein molecule allows the use of much harsher derivatization conditions than many proteins can sustain, is relatively inexpensive, and often yields a linkage that is stable to a wide range of denaturing conditions. A number of derivatized beads are commercially available, all with various constituents and sizes. Beads formed from synthetic polymers such as polyacrylamide, polyacrylate, polystyrene, or latex are commercially available from numerous sources such as Bio-Rad Laboratories (Richmond, Calif.) and LKB Produkter (Stockholm, Sweden). Beads formed from natural macromolecules and particles such as agarose, crosslinked agarose, globulin, deoxyribose nucleic acid, and liposomes are commercially available from sources such as Bio-Rad Laboratories, Pharmacia (Piscataway, N.J.), and IBF (France). Beads formed from copolymers of polyacrylamide and agarose are commercially available from sources such as IBF and Pharmacia. Magnetic beads are commercially available from sources such as Dynal Inc. (Great Neck, N.Y.).

Brief Summary Text (11):
Microparticles prepared using lipids to encapsulate target drugs are currently available. For example, lipids arranged in bilayer membranes surrounding multiple aqueous compartments to form particles may be used to encapsulate water soluble drugs for subsequent delivery as described in U.S. Pat. No. 5,422,120 to Sinil Kim. These particles are generally greater than 10 .mu.m in size and are designed for intraarticular, intrathecal, subcutaneous and epidural administration. Alternatively, liposomes have been used for intravenous delivery of small molecules. Liposomes are spherical particles composed of a single or multiple phospholipid and cholesterol bilayers. Liposomes are 30 .mu.m or greater in size and may carry a variety of water-soluble or lipid-soluble drugs. Liposome technology has been hindered by problems including purity of lipid components, possible toxicity, vesicle heterogeneity and stability, excessive uptake and manufacturing or shelf-life difficulties.

Drawing Description Text (8):
FIG. 7 is a bar graph showing the amount of expressed gene product by .beta.-Galactosidase activity in milliunits versus microparticle formation for naked DNA, cationic liposomes containing DNA, and DNA microparticles.

Detailed Description Text (20):
When preparing microparticles containing protein, a protein stabilizer such as glycerol, fatty acids, sugars such as sucrose, ions such as zinc, sodium chloride, or any other protein stabilizers known to those skilled in the art may be added prior to the addition of the polymers during microparticle formation to minimize protein denaturation.

Detailed Description Text (123):
The amount of expressed .beta.-galactosidase enzyme from lysed cells that were

transfected using either: 1) naked DNA (no addition); 2) cationic liposomes plus DNA; or 3) DNA-containing microparticle, prepared as described above, is shown in FIG. 7.

L10: Entry 60 of 143 File: USPT May 15, 2001

DOCUMENT-IDENTIFIER: US 6232085 B1
** See image for Certificate of Correction **
TITLE: Method for determining conditions that stabilize proteins

Brief Summary Text (19):
When bacterial cells are used to overexpress exogenous proteins, the recombinant protein is often sequestered in bacterial cell inclusion bodies. For the recombinant protein to be useful, it must be purified from the inclusion bodies. During the purification process, the recombinant protein is denatured and must then be renatured. It is impossible to predict the renaturation conditions that will facilitate and optimize proper refolding of a given recombinant protein. Usually, a number of renaturing conditions must be tried before a satisfactory set of conditions is discovered. In a study by Tachibana et al., each of four disulfide bonds were singly removed, by site-directed mutagenesis, from hen lysozyme (Tachibana et al., Biochemistry 33:15008-15016 (1994)). The mutant genes were expressed in bacterial cells and the recombinant proteins were isolated from inclusion bodies. Each of the isolated proteins were renatured under different temperatures and glycerol concentrations. The efficacy of protein refolding was assessed in a bacteriolytic assay in which bacteriolytic activity was measured as a function of renaturing temperature. The thermal stability of each protein was studied using a physical thermal shift assay. In this study, however, only one sample reaction was heated and assayed at a time. The single sample heating and assay configuration prevents the application of thermal shift technologies to high throughput screening of a multiplicity of protein refolding conditions. Thus, there is a need for a thermal shift technology which can be used to rank the efficacies of various protein refolding conditions.

Detailed Description Text (289):
The increased yield of refolded protein at pH 8.9 and 7% glycerol was found to be 17.8%, 32% higher than the yield obtained at a pH 8.0 and 0.5 M NaCl (13.5.+-.0.2% yield). The near additivity of refolding determinants has important consequences since it suggests that the small individual free energy components that comprise the overall free energy of folding can be incrementally combined to optimize the yield of folded protein.

Detailed Description Text (290):
Results of a second round of refolding experiments at a final Gdn-HCl concentration of 0.09 M revealed that the Gdn-HCl is an even more important factor affecting the folding of His.sub.6 -D(II)FGFR1 (Table 9). At pH 8.0 and 0.5 M NaCl, decreasing the Gdn-HCl concentration to 0.09 M doubled the refolded protein yield, relative to the yield obtained at pH 8.0, 0.5 M NaCl, and 0.38 M Gdn-HCl (Table 9). In accordance with the results obtained at a Gdn-HCl concentration of 0.38 M, the yield of refolded His.sub.6 -D(II)-FGFR1 in 0.09 M Gdn-HCl was also increased in the presence of glycerol. These results suggest that the improved yield of refolded His.sub.6 D(II)-FGFR1 in glycerol (5 to 10%) and lower Gdn-HCl concentration are additive. Further, the results in Table 9 reveal that the Hofmeister salt Na.sub.2 SO.sub.4 increases the yield of refolded protein almost as well as 5 to 10% glycerol.

Other Reference Publication (62):

Smirnov, O.N., et al., "Study on the Interaction of the cholera toxin and its B-subunit with liposomes containing ganglioside GM1 and fluorescent-labeled gangliosides," Biologicheskie Membrany 12: 174-184 (1995).

Other Reference Publication (64):
Surin, A.M., et al., "A study of the interaction of cholera toxin B-subunit with liposomes containing ganglioside GM1 and fluorescein-labeled lipids," Biologicheskie Membrany 9: 495-508 (1992).

L10: Entry 93 of 143 File: USPT Mar 2, 1999

DOCUMENT-IDENTIFIER: US 5876992 A
TITLE: Method and formulation for stabilization of enzymes

Brief Summary Text (8):
Excipients are inert substances that give a desired characteristic to solvents. Examples of such excipients include sugars, glycerol, polyethylene glycols, amino acids, and other osmolytes. Certain hydrophilic excipients confer stability by making the solvent more polar. An increase in solvent polarity results in an increase in the free energy of transfer for the hydrophobic amino acid moieties from within the protein to the solvent thereby making it more difficult for the protein to unfold (Alonso and Dill, 1991). Glycerol is a common excipient used by enzymologists for storage of enzymes at low temperatures (-20.degree. C.), since some enzymes stored frozen in 50% glycerol buffers may retain much of their initial activity for years.

Brief Summary Text (12):
The use of sugars such as the disaccharide trehalose as stabilizing agents has been used in conjunction with lyophilization to stabilize polysaccharides (Guthohrlein and Helting, European Patent No. GB 2 009 198 A) and liposomes (Crowe et al., 1987). Lyophilization has also been used to stabilize tumor necrosis factor in the presence of a nonionic surfactant and sugars such as trehalose (Hayashi and Komiya, European Patent No. GB 2 126 588 A). Enzymes that have been stabilized with sugars during lyophilization include phosphofructokinase (Carpenter et al., 1987), which was stabilized with glucose, galactose, maltose, sucrose, and trehalose, and alkaline phosphatase (Ford and Dawson, 1992) which was stabilized with mannitol, lactose, and trehalose.

Detailed Description Text (5):
The purified enzymes that were used to study potential stabilizing excipients were T7 RNA Polymerase, Avian Myeloblastosis Virus-Reverse Transcriptase (AMV-RT) and RNase H (Molecular Biology Resources, Milwaukee, Wis.). These enzymes were stored in 50% glycerol storage buffers at -20.degree. C. or -70.degree. C., since glycerol lowers the freezing point of proteins closer to the storage temperature. Before lyophilization, it was necessary to remove glycerol because it acts as a hygroscopic agent and the enzyme preparations would not lyophilize. Each enzyme was individually deglycerolized by ultrafiltration into its appropriate storage buffer containing no glycerol as described below.

Detailed Description Text (9):
To deglycerolize the enzymes, an appropriate volume of enzyme (in glycerol) was added to each concentrator. The amount that was deglycerolized was determined by the number of units required in the final lyophilization. Generally, an amount from 50-150 .mu.L enzyme in glycerol buffer was added to the prepared concentrators. The appropriate buffer without glycerol was added to obtain a volume of about 10 times the initial enzyme volume (up to 1.5 ml total) and the two phases were thoroughly mixed by pipetting, being careful not to disrupt the filter. It is important to avoid overdilution of the enzymes with the non-glycerol buffer, since this may contribute to activity loss. Each concentrator was centrifuged in an SA-600 rotor at 5000.times.g for approximately 45 minutes. The process was repeated twice.

Detailed Description Text (64):
Crowe, J. H., B. J. Soargo, and L. M. Crowe. Preservation of dry liposomes does not require retention of residual water. Proc. Natl. Acad. Sci., 84: 1537-1540 (1987).

Other Reference Publication (5):
Crowe et al., "Preservation of Dry Liposomes Does Not Require Retention of Residual Water" Proc. Natl. Acad. Sci., 84:1537-1540 (Mar. 1987).

L10: Entry 101 of 143 File: USPT Nov 10, 1998

DOCUMENT-IDENTIFIER: US 5834254 A
TITLE: Stabilized enzyme compositions for nucleic acid amplification

Detailed Description Text (6):
The enzyme preparation was stored at -20.degree. C. in a storage buffer containing 20-50 mM Tris-HCl (pH 7.5), 0.1M NaCl, 0.1 mM ethylenediamine tetraacetic acid (EDTA), 1.0 mM dithiothreitol (DTT), 0.01% (v/v) TERGITOL NP.RTM.-40 (TERGITOL NP.RTM. is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.) or 0.1% (v/v) TRITON.RTM. X-100 (TRITON.RTM. is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.), and 50% (v/v) glycerol. Purified T7 RNA polymerase was obtained from Epicentre Technologies, Madison, Wis. Prior to dialysis the enzyme was stored in 50% (v/v) glycerol, 50 mM Tris-HCl (pH 7.5), 0.1M NaCl, 1.0 mM DTT, 0.1 mM EDTA and 0.1% (v/v) TRITON.RTM. X-100. This enzyme was also stored at -20.degree. C. prior to dialysis.

Other Reference Publication (11):
Crowe, John H. and Crowe, Lois M., Factors affecting the stability of dry liposomes. Biochimica et Biophysica Acta 939:327-334 (1988).